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THE EFFECTS OF STIMULATION OF THE CAROTID BODY CHEMORECEPTORS ON THE PULMONARY VASCULAR BED IN THE DOG: THE 'VASOSENSORY CONTROLLED PERFUSED LIVING ANIMAL' PREPARATION

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We have shown previously that stimulation of the carotid body chemoreceptors by venous blood usually caused a reflex decrease in pulmonary vascular resistance in lungs perfused at a constant blood volume inflow (Daly & Daly, 1957*b*). It was stated that the method used did not enable us to establish whether this effect was a direct reflex response of the pulmonary vascular bed proper, i.e. pulmonary vasodilatation, or was passive to reflex bronchial vasomotor changes causing an alteration in the distribution of blood between the bronchial and pulmonary vascular systems (Berry & I. de B. Daly, 1931). The only way of distinguishing between these two possibilities was to repeat the tests when no blood was flowing through the bronchial circulation. This paper describes, first, the experimental procedure carried out to achieve the necessary conditions to obtain unequivocal evidence for a reflex acting on the pulmonary vascular bed proper, and secondly, the results of an investigation carried out to determine whether the carotid body chemoreceptors exert any reflex control over this vascular bed.

The perfused living animal preparation in which the blood flow through the greater and lesser circulations was under independent control went some way to meet the necessary requirements (I. de B. Daly, Elsden, Hebb, von Ludány & Petrovskaia, 1942; I. de B. Daly, Eggleton, Hebb, Linzell & Trowell, 1954). It was necessary, however, to modify the perfusion of the systemic circulation in this preparation for the following reasons. (1) When making tests of chemoreceptor stimulation in the absence of the bronchial circulation, i.e. at zero bronchial arterial pressure, the blood pressure in other parts of the

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systemic circulation had to be maintained. For this reason the thoracic aorta, from which the bronchial arteries arise at levels T4–T8 (Berry, Brailsford & I. de B. Daly, 1931; Notkovich, 1957), was separately perfused. (2) Account also had to be taken of the fact that changes in systemic blood pressure themselves cause alterations in pulmonary vascular resistance reflexly from the carotid sinus baroreceptors (Daly & Daly, 1957*a*). Thus it was necessary to have under control the pressure not only in the carotid sinuses but also in the aortic arch which itself might exert a reflex effect on the pulmonary vascular resistance. These conditions required separate perfusion of the carotid bifurcation areas and of the aortic arch, whereby the pressure in each vasosensory area could be controlled. (3) Since constant conditions in the central connexions of the reflex arc had to be ensured, the cerebral circulation was separately perfused. (4) In order to exclude reflex effects occurring as a result of changes in pressure in the vascular bed of the abdominal aorta, this territory was also separately perfused.

It will be seen from the design of the perfusion systems (Fig. 1) that the gaseous composition of the blood perfusing each separate part of the systemic circulation was the same, with the exception of the blood to the carotid body chemoreceptors, the composition of which could be changed at will. The composition of the venous blood perfusing the pulmonary circulation remained constant. Thus, it will be evident that the reflexogenic areas were under separate control as well as the cerebral circulation, the remaining part of the systemic circulation and the pulmonary circulation. For these reasons we propose to call this kind of perfusion of the whole animal the 'vasosensory controlled perfused living animal' preparation.

We were also concerned with another consideration, namely, that having excluded all known *passive* mechanisms affecting the pulmonary vascular bed, there remained the possibility that the observed *active* response may have been the result not of a primary reflex but of a secondary or even a tertiary reflex or humoral mechanism which was prepotent. Such a separation of a chain of biological events can be achieved in the 'vasosensory controlled perfused living animal' by maintaining constant the blood pressure in the various vascular territories under separate perfusion, with the exception of that which is under test for direct reflex vasomotor control. In practice this is achieved by compensating any change in perfusion pressure by adjustments to the output volumes of the perfusion pumps.

METHODS

Dogs varying in weight from 10·8 to 15·5 kg were anaesthetized with chloralose (0·1 g/kg intravenously) after premedication with morphine hydrochloride (1 mg/kg subcutaneously). They were ventilated by means of a Starling 'Ideal' pump at a constant peak inflationary pressure which varied between 60 and 110 mm H_2O in different experiments (Konzett & Rössler, 1940). During

expiration the lungs collapsed passively against a resistance of 20–30 mm H_2O. Both phrenic nerves were crushed to minimize mechanical effects on the lungs.

The method of simultaneous and separate perfusion of the systemic and pulmonary circulations was similar to that described by I. de B. Daly *et al.* (1942), and I. de B. Daly *et al.* (1954), but a modification was made in that both carotid bifurcation regions and also the aortic arch were isolated from the circulation and separately perfused. In regard to the remainder of the systemic circulation, the head, neck and upper limbs on the one hand and the vascular bed supplied by the descending aorta on the other were separately perfused.

Perfusion of the carotid bodies and sinuses

Reflex effects from the carotid bodies were elicited by temporarily changing the perfusate either from oxygenated blood to venous blood or from oxygenated blood to hypoxic blood. For this purpose both carotid bifurcation regions were isolated from the circulation by ligation of all branches of the common and external carotid arteries. The veins draining the carotid bodies were preserved (Chungcharoen, M. de B. Daly & Schweitzer, 1952). The carotid bodies were perfused through the common carotid arteries by means of a Sigmamotor pump (Sigmamotor, Inc. New York) with blood from a donor dog (No. 1) anaesthetized with morphine (2 mg/kg subcutaneously) and a mixture of chloralose (0·05 g/kg) and urethane (0·5 g/kg intravenously). The input side of the pump (P_1 in Fig. 1) was connected by means of a three-way tap *a* to the carotid artery of the donor, and to the right auricle via a cannula inserted through the right external jugular vein. The output side of the pump was connected through a warming coil (*c*) to the peripheral ends of the common carotid arteries of the recipient animal. Blood from the cannulated external carotid arteries was returned to a vein of the donor dog via a Starling type of resistance *b* by means of which the pressure in the carotid sinuses could be either maintained constant or altered at will. The carotid sinus pressure was measured with a mercury manometer.

A change-over of perfusate from oxygenated to venous blood was achieved by turning the tap *a* through 180° from the position shown in Fig. 1 whilst the donor animal breathed room air. A change-over from oxygenated to hypoxic blood was achieved by changing the ventilation of the donor dog from room air to a gas mixture containing 5% O_2 and 5% CO_2 in N_2 whilst the tap *a* remained in the same position as that shown in Fig. 1.

Perfusion of the systemic circulation

The vascular bed of the descending thoracic aorta

The aorta immediately beyond the origin of the left subclavian artery was tied and the vascular bed of the descending thoracic aorta which included the bronchial circulation was perfused, via T-cannulae inserted into the femoral arteries, by means of a Dale–Schuster pump (P_2). The perfusion pressure was measured with a mercury manometer.

The aortic arch was isolated from the circulation by tying, in addition to the ligature beyond the left subclavian artery, the ascending aorta just above the origin of the coronary arteries, the brachiocephalic and left subclavian arteries. The pressure in the aortic arch was controlled by means of a pressure-bottle filled with blood and connected to a cannula inserted into the left subclavian artery. The aortic arch pressure was therefore non-pulsatile; it was measured with a Frank membrane manometer.

An alternative method of perfusion of the aortic arch is shown in Fig. 2*A* in which the arch pressure was identical with the cerebral arterial pressure (see below). In these experiments the brachiocephalic artery was not ligated. For the purpose of carrying out tests of chemoreceptor stimulation at zero bronchial arterial pressure when using either of these methods of aortic perfusion, the pump perfusing the vascular bed of the descending thoracic aorta was switched off.

In a second alternative method in which the aortic arch was perfused by the cerebral perfusion pump, the aorta was tied immediately above the diaphragm instead of beyond the left subclavian artery (Fig. 2*B*). When it was necessary to perform tests of chemoreceptor stimulation during

temporary interruption of the bronchial circulation, the bronchial arterial and aortic arch pressures were reduced to zero—without affecting the cerebral perfusion pressure—by transferring a clamp at point *a* (Fig. 2*B*) to point *b*.

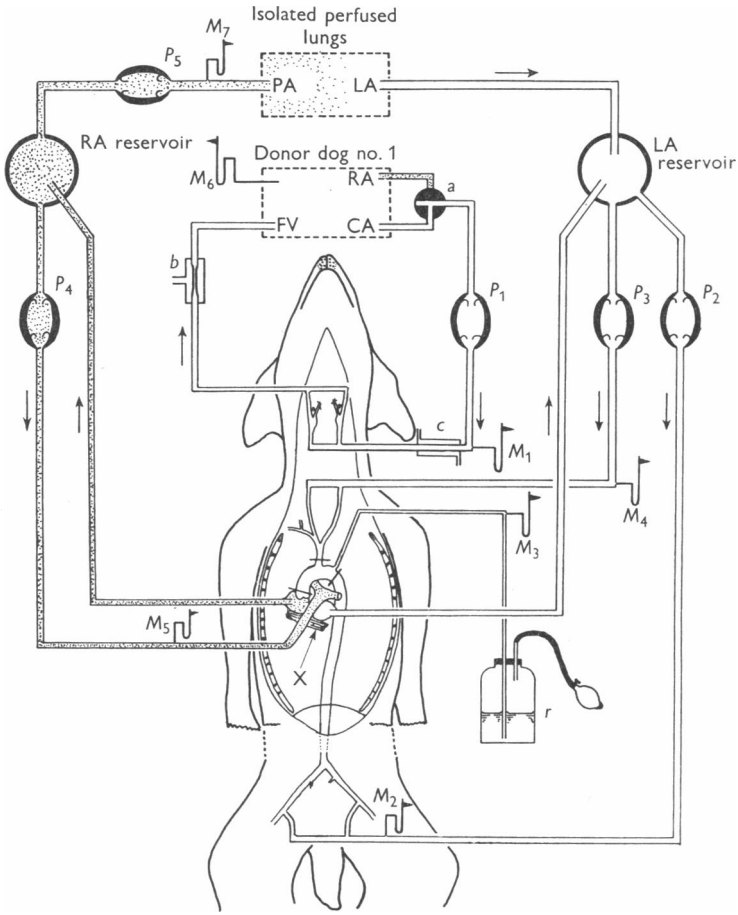
The cerebral circulation

The head and right upper limb were perfused, via cannulae inserted into the *central* ends of the common carotid arteries, by a Dale–Schuster pump (P_3 in Fig. 1). The pressure was measured with a mercury manometer. The brain therefore received its main blood supply via the right vertebral artery.

The input sides of pumps P_2 and P_3 were connected to the left auricle reservoir containing oxygenated blood. The blood after perfusing the systemic circulation returned through natural channels to the right auricle, the appendix of which was cannulated, and from thence into the right auricle reservoir (Fig. 1, *RA*).

Perfusion of the lungs

The lungs were perfused at constant blood volume inflow with venous blood from the right auricle reservoir by means of a Dale–Schuster pump (P_4). The pulmonary arterial cannula was



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passed through the wall of the right ventricle and tied into the main pulmonary artery. In addition, a tape which gripped the pulmonary arterial cannula was tied round the ventricles immediately below the auriculo-ventricular groove, thereby putting the ventricles out of action. The left auricle was cannulated and blood returning from the lungs passed freely through wide-bore rubber tubing into the left auricle reservoir (*LA*). Pulmonary arterial pressure was measured by means of a Marey tambour.

Equilibration of blood

Another donor dog (No. 2) received morphine hydrochloride (1–2 mg/kg subcutaneously) and was bled to death from a femoral artery under local anaesthesia. The blood was heparinized (Liquemin, Roche Products Ltd.) on the basis of 35–40 mg/kg. One-third of this dose was given intravenously before bleeding, the remainder being added to the shed blood. The lungs were removed from the body, transferred to a warmed Perspex tray and perfused through the pulmonary artery with blood drawn from the right auricle reservoir by means of a Dale–Schuster pump (P_5). For equilibration of the blood in the reservoirs with O_2 and CO_2, the blood was circulated through these lungs, ventilated with 7% or 10% CO_2 in O_2, for about 1 hr before perfusion of the recipient animal was begun. Subsequently the perfusion system of these lungs was put in parallel with the perfused lungs of the recipient animal, as is shown in Fig. 1.

The blood of donor dog No. 1 and of the recipient was rendered incoagulable with heparin (35–40 mg/kg body wt.). Two and sometimes three other dogs were bled to death from a femoral artery under local anaesthesia the evening before. The blood was heparinized and was used to fill

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Legend to Fig. 1

Fig. 1. The 'vasosensory controlled perfused living animal preparation'. Diagram showing the methods of perfusion of the vasosensory areas of the carotid bifurcations and aortic arch, of the brain, the remaining parts of the systemic circulation and the lungs; the lungs are not shown. The isolated carotid sinuses and bodies are perfused by pump P_1 via tap (*a*) with either oxygenated blood from the carotid artery (*CA*) or venous blood from the right auricle (*RA*) of donor dog No. 1. Blood from the cannulated external carotid arteries is returned to the donor animal via a femoral vein. The pressure in the carotid sinuses is controlled by the Starling type of resistance (*b*). The aortic arch is isolated from the circulation by tying the ascending aorta, the brachiocephalic and left subclavian arteries and the aorta immediately beyond the origin of the left subclavian artery. The pressure in the aortic arch is controlled by means of a pressure bottle (*r*) filled with blood and connected to a cannula inserted into the left subclavian artery.

The brain is perfused by pump P_3 with blood from the left auricle reservoir (*LA*) via cannulae inserted into the common carotid arteries, pointing caudally. The vascular bed of the aorta beyond the origin of the left subclavian artery is perfused by pump P_2 via T-cannulae inserted into the femoral arteries. Blood from the systemic circulation drains via a cannula in the right auricle into the right auricle reservoir (*RA*). The lungs of the recipient animal are perfused through the pulmonary artery with venous blood from the right auricle reservoir by means of pump P_4. Blood from the cannulated left auricle drains into the left auricle reservoir. Connected in parallel with the lungs of the recipient animal are the isolated lungs of donor dog No. 2. These lungs are perfused through the pulmonary artery by pump P_5 with blood from the right auricle reservoir; blood from the left auricle drains into the left auricle reservoir. M_1, M_2, M_3, M_4, M_5, M_6 and M_7 are connexions to manometers measuring pressures in the different vascular territories. CA, carotid artery; FV, femoral vein; LA, left auricle; RA, right auricle; PA, pulmonary artery; *a*, three-way tap; *b*, Starling type of resistance; *c*, warming coil; *X*, tape tied round ventricles below auriculo-ventricular groove and embracing the pulmonary arterial cannula. The stippling represents venous blood. For further details see text.

the perfusion apparatus. All perfusion pumps and venous reservoirs were immersed in a thermostatically controlled water-bath maintained at a constant temperature of 37–40° C in different experiments.

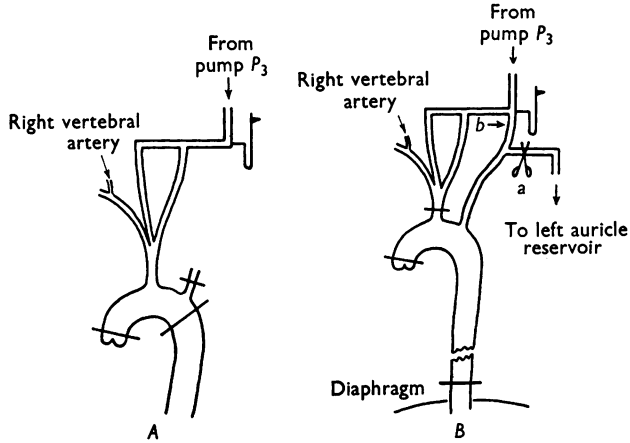

Fig. 2. Modified methods of perfusion of the aortic arch. *A*. Isolated aortic arch perfused from the cerebral perfusion pump (P_3 in Fig. 1) via the brachiocephalic artery. Remainder of perfusion systems as in Fig. 1. *B*. Aortic arch and thoracic aorta as far as level of the diaphragm perfused by cerebral perfusion pump (P_3 in Fig. 1). Pressure in the aorta (bronchial arteries) was reduced to zero by removing clamp at *a* and applying it at *b*. Remainder of perfusion systems as in Fig. 1. For details see text.

Blood analyses

Blood samples were taken from the recipient and donor animals during the course of each experiment. Oxygen and carbon dioxide contents were determined manometrically by the method of Van Slyke & Neill (1924). Duplicate values agreed to within 0·2 ml./100 ml. Haemoglobin was determined photometrically as cyanmethaemoglobin by the method of Stadie (1920) as modified by Wu (1922). Oxygen capacity was calculated from the haemoglobin concentration, on the assumption that 1 g haemoglobin combines with 1·34 ml. oxygen.

Estimations of blood pH were performed anaerobically at room temperature with a Stadie glass electrode and either a Pye (Model 605, Pye Instrument Co.) or a Vibron (Model C 33 B, Electronic Instruments, Ltd.) pH meter. The apparatus was standardized before each determination by means of phosphate buffers of pH 7·2 and 7·4 at 20° C. The accuracy between duplicate samples was 0·01 pH units or better when estimations were carried out with either of the pH meters. In a few experiments each blood sample was determined in duplicate, using both pH meters, and the order of accuracy of the four estimations was again found to be the same. Measurements of blood pH made at ambient temperature were converted to the corresponding values of 37° C by using the temperature coefficient of Rosenthal (1948).

Carbon dioxide tension was estimated from the observed values for carbon dioxide content, pH, haemoglobin content and percentage oxygen saturation, using the formula:

$$pCO_2 = \frac{CO_2}{S(10^{(pH-pK')}+1)}$$

(Severinghaus, Stupfel & Bradley, 1956*a*). The plasma CO_2 content (mM) was calculated by multiplying the value for whole blood by the factor '*f*' read from the nomogram of Van Slyke & Sendroy (1928). The solubility factor *S* was read from the table of Severinghaus *et al.* (1956*a*), and the value for pK′ from the nomogram of Severinghaus, Stupfel & Bradley (1956*b*).

Order of operative procedures

The 'vasosensory controlled perfused living animal' preparation was made in four stages as follows:

(1) The recipient and donor dog No. 1 were anaesthetized. The carotid bifurcation regions of the recipient animal were prepared for perfusion. At the same time the following blood vessels of the donor dog were cannulated: a carotid artery for connexion to tap *a*, a femoral artery for measurement of blood pressure (M_6), the right external jugular vein for passage of a cannula into the right auricle for connexion to tap *a*, and a femoral vein for return of blood from the carotid sinuses of the recipient animal.

(2) Artificial ventilation was then applied to the recipient animal and its chest opened by splitting the sternum in the mid line. After ligation of the internal mammary vessels, loose ligatures were placed on the following vessels: the main pulmonary artery, the ascending aorta, the aortic arch immediately beyond the subclavian artery, the brachiocephalic and left subclavian arteries. Meanwhile, donor dog No. 2 was bled to death from a femoral artery and after cannulation of the pulmonary artery and opening the left auricle to allow escape of blood during perfusion, its lungs were removed and transferred to the Perspex tray. The reservoirs and all perfusion pumps were filled with blood and perfusion of the now isolated lungs of donor dog No. 2 was begun for equilibration of the blood in the extracorporeal circulation.

(3) Isolation of the carotid bifurcation regions of the recipient animal was completed by inserting cannulae in the common carotid arteries, pointing cranially, and in the external carotid arteries, pointing caudally. The connexions previously described were then made with donor dog No. 1 and perfusion of the carotid sinuses and bodies begun.

(4) The central ends of the common carotid and also the femoral arteries of the recipient animal were cannulated and connected to pumps P_3 and P_2, respectively. Wide-bore cannulae were tied in the left and right auricles and connected to their respective venous reservoirs. The change-over of the recipient animal's natural circulation to separate perfusion of the systemic and pulmonary circulations was then carried out (for details of this procedure, see I. de B. Daly *et al.* 1942).

(5) Having now established perfusion of the systemic circulation through the common carotid and femoral arteries, the aortic arch was isolated from the circulation by tying the ligatures on the ascending aorta, the aorta beyond the origin of the left subclavian artery or immediately above the diaphragm, and the brachiocephalic artery. The left subclavian artery was also ligated, a cannula being inserted into its caudal end and connected to the reservoir of blood (Fig. 1, *r*).

The outputs of the two systemic pumps were adjusted initially so that the perfusion pressures lay between 100 and 140 mm Hg. Likewise the pulmonary arterial pressure was adjusted to 15–26 cm saline.

RESULTS

Gaseous composition of the perfusate

It has been shown previously in the perfused living animal that the pH values of arterial blood are more acid than can be accounted for in terms of concentration of carbon dioxide in the blood, a fact which was attributed to the high blood lactate concentrations (I. de B. Daly *et al.* 1954). In the present series of experiments the values for pH and carbon dioxide content were low, no doubt owing to a high blood lactate concentration, but the oxygen saturation of the blood was within normal limits (Table 1). These findings are not inconsistent with a metabolic acidosis.

The carotid body chemoreceptors were stimulated by changing the perfusate either from oxygenated to venous blood or from oxygenated to hypoxic blood.

The changes in O_2 and CO_2 tension and in pH of the carotid body perfusate supplied by donor dog No. 1 are shown in Table 2.

TABLE 1. Values for the oxygen saturation, pO_2, pCO_2 and pH of the arterial blood perfusing the 'vasosensory controlled perfused living animal' preparation

Expt. No.	Time* (min)	O_2 saturation (%)	pO_2 (mm Hg)	pCO_2 (mm Hg)	pH
1	35	97	100	43	7·14
2	38	90	67	37	7·25
3	24	95	90	43	7·15
4	70	94	92	73	6·96
5	60	82	55	43	7·12
6	63	79	63	68	7·09
7	50	88	78	72	6·97
8	41	89	72	52	7·16
9	97	98	110	54	6·97
11	22	95	88	49	7·17
12	61	95	85	42	7·19

* Time after start of perfusion.

TABLE 2. The values for pO_2, pCO_2 and pH of the blood perfusing the carotid bodies. The carotid bodies were stimulated by changing the perfusate either from oxygenated to hypoxic blood (*A*) or from oxygenated to venous blood (*B*)

	No. of expts.	No. of observations		pO_2 (mm Hg)	pCO_2 (mm Hg)	pH
A	3	5	Oxygenated blood	105 (87–110)	40·8 (36–43)	7·20 (7·13–7·25)
			Hypoxic blood	25·6 (22–33)	38·0 (34–45)	7·28 (7·25–7·32)
B	6	9	Oxygenated blood	73·0 (54–92)	42·3 (37–54)	7·20 (7·11–7·30)
			Venous blood	43·1 (33–56)	53·2 (44–69)	7·18 (7·07–7·30)

The open figures indicate the mean values, those in parentheses the range.

Effects of stimulation of the carotid body chemoreceptors on pulmonary vascular resistance

It has been shown previously (Daly & Daly, 1957*b*) in the entire animal in which one or more lung lobes were ventilated and perfused under controlled conditions that stimulation of the carotid body chemoreceptors by venous blood caused a decrease in pulmonary vascular resistance in seven experiments and an increase in one. In these experiments the bronchial circulation was intact.

In the present experiments tests of carotid body chemoreceptor stimulation were made under the following conditions: (1) with the bronchial circulation perfused at normal levels of systemic arterial pressure; (2) with the bronchial arterial pressure reduced to zero, and (3) with the bronchial arterial pressure zero and at the same time the pressures in the remaining parts of the systemic circulation compensated against reflex changes. It should be pointed out that,

although the bronchial circulation is necessary for maintaining the viability of the pulmonary vasomotor nerves in the dog (I. de B. Daly & von Euler, 1932), pulmonary vasomotor responses to electrical stimulation of nerves to the lungs can be obtained during cessation of the bronchial circulation for periods of 5–10 min (I. de B. Daly, Duke, Hebb & Weatherall, 1948; P. R. Allison & I. de B. Daly, unpublished observations). In the present experiments the periods of zero bronchial arterial pressure during tests of chemoreceptor stimulation did not exceed 5 min. It was again found that chemoreceptor stimulation caused changes in pulmonary arterial perfusion pressure. These changes in pressure, as in our previous experiments, can be evaluated in terms of changes in pulmonary vascular resistance, since the pulmonary arterial blood inflow volume and the left auricular pressure were maintained constant.

TABLE 3. The effects of stimulation of the cartoid body chemoreceptors by venous or hypoxic blood on pulmonary vascular resistance with and without circulation of blood through the bronchial vascular system

Expt. No.	Method of aortic arch perfusion; as in Fig.	Response of pulmonary vascular resistance: Bronchial circulation intact	Response of pulmonary vascular resistance: Zero bronchial arterial B.P.
2	2*A*	+	0
3	1	−	+
4	1	+	+
6	1	0	+*
8	1	−	.
9	1	−	+
12	2*B*	−	+

+, −, 0 = increase, decrease, no change in pulmonary vascular resistance, respectively.
* Response abolished by bilateral stellectomy.

All the observed changes in pulmonary vascular resistance occurred independently of alterations in ventilation overflow volume and were therefore not passive to bronchomotor events. Furthermore, the pulmonary vascular resistance might have been influenced mechanically by the accompanying reflex increase in rhythmic movements of the thoracic cage. In all experiments this possibility was excluded by preventing such movements with decamethonium iodide (Geigy, or Light and Co., 0·25 mg/kg intravenously).

It was found that when blood was circulating through the bronchial vascular system stimulation of the carotid body chemoreceptors resulted in a decrease in pulmonary vascular resistance in four experiments (Fig. 3*A*), an increase in two (Fig. 4*A*) and no change in one. These results are summarized in Table 3. Similar effects were observed whether the chemoreceptors were stimulated by venous or by hypoxic blood; they were reversed when oxygenated blood perfusion of the carotid bodies was restored.

The outstanding feature of these experiments was that, irrespective of the direction of change in the pulmonary vascular resistance caused by chemoreceptor stimulation when blood was circulating through the bronchial circulation, there invariably occurred an increase in pulmonary vascular resistance when a similar test was carried out at zero bronchial arterial pressure. Thus in three of the four experiments in which the initial response was a reduction in pulmonary vascular resistance, an increase now occurred (Expts. 3, 9 and 12 in Table 3). In the two experiments in which an increase in pulmonary vascular resistance occurred with the bronchial circulation intact, the response persisted at zero bronchial artery pressure in one, and disappeared permanently in the other (Expts. 4 and 2 in Table 3). In Expt. 6, in which no change in pulmonary vascular resistance occurred with the bronchial circulation intact, an increase in vascular resistance appeared when the test was repeated at zero bronchial arterial pressure. In Expt. 8 a test at zero bronchial arterial pressure was not made.

Figure 3 is taken from an experiment in which the initial response to stimulation of the carotid body chemoreceptors by venous blood with the bronchial circulation intact was a decrease in pulmonary arterial perfusion pressure, indicating a decrease in pulmonary vascular resistance (*A*). When the test was repeated at zero bronchial arterial pressure, which in this experiment was brought about by switching off the pump perfusing the vascular bed of the aorta caudal to the origin of the left subclavian artery, an increase in pulmonary vascular resistance occurred (*B*). Since these tests were made under conditions which exclude all other known passive effects, i.e. cardiac, respiratory, bronchomotor and left auricular pressure changes (see I. de B. Daly, 1956), it is concluded that carotid body stimulation causes reflex constriction of the pulmonary vascular bed proper.

This vasoconstriction is the result of a *primary* reflex effect from the carotid bodies and is not dependent upon secondary reflexes or upon secretion of hormones formed elsewhere in the body. The evidence for this is as follows: (1) In some tests of stimulation of the carotid bodies, secondary reflex effects arising from concomitant changes in pressure in various vascular territories of the systemic circulation were prevented by compensation. This was achieved by adjustments to the output volumes of the pumps perfusing these vascular territories. It was found that the increase in pulmonary vascular resistance occurring at zero bronchial arterial pressure persisted under such conditions in which the pressures in the cerebral circulation, carotid sinuses, aortic arch and in the vascular bed of the descending thoracic aorta were maintained constant (Fig. 3). (2) The pO_2, pCO_2 and pH of the blood perfusing the whole of the systemic circulation, with the exception of the carotid bodies, was unchanged during tests of chemoreceptor stimulation, and changes in gaseous composition of the blood could not, therefore, have been responsible for the

observed pulmonary vasomotor responses. (3) The pulmonary vascular responses might have been due to a reflex alteration in the rate of secretion of suprarenal medullary hormones. This was excluded because the responses still occurred during temporary cessation of perfusion of the vascular bed of the descending thoracic aorta. In tests of chemoreceptor stimulation carried out under these conditions, no hormones from the suprarenal glands could have gained access to the right auricle reservoir, blood from which is perfused through the lungs.

We interpret our results as indicating that, when tests of chemoreceptor stimulation are made with the bronchial circulation intact, the change in

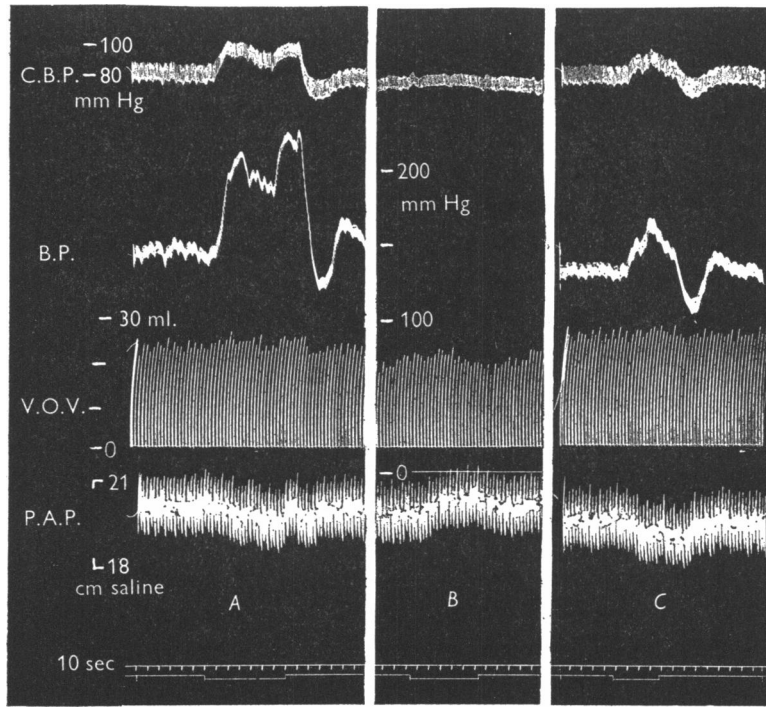

Fig. 3. Dog, 11·6 kg. Morphine-chloralose. 'Vasosensory controlled perfused living animal' preparation. The aortic arch was separately perfused as shown in Fig. 1 and the pressure was maintained at 60 mm Hg. The mean carotid sinus pressure was maintained constant at 100 mm Hg. In *A*, *B* and *C*, the carotid body chemoreceptors were stimulated temporarily by venous blood from the right auricle of donor dog No. 1. In *B*, the test was made at zero bronchial arterial pressure, the pump perfusing the vascular bed of the aorta beyond the origin of the left subclavian artery being turned off. The cerebral perfusion pressure was maintained constant. Note reversal of the response of the pulmonary arterial pressure in *B*. In this and in Fig. 4, B.P., femoral arterial blood pressure; C.B.P., cerebral blood pressure; C.S.P., carotid sinus pressure; P.A.P., pulmonary arterial pressure; V.O.V., ventilation overflow volume. Time marker, 10 sec.

pulmonary vascular resistance depends upon the algebraic sum of two effects: first, haemodynamic changes in the bronchial circulation which result in a passive alteration in pulmonary vascular resistance due to reflex changes either in systemic (bronchial) arterial pressure or in bronchial vascular resistance (I. de B. Daly, 1956); and secondly, a direct *reflex* effect on the pulmonary vascular bed proper causing an *active* increase in pulmonary vascular resistance.

An attempt was made to trace the nervous pathways involved in the pulmonary vascular reflex, and in one experiment it was found that the increase in pulmonary vascular resistance occurring at zero bronchial arterial pressure on stimulation of the chemoreceptors was abolished by destruction of both stellate ganglia (Table 3). In other experiments the response disappeared before the crucial test could be carried out.

The effects of the bronchial circulation on pulmonary vascular resistance

Because between the systemic and pulmonary circulations there are communications via the bronchial system of vessels, a change in either systemic pressure or in resistance of the bronchial arteries and their communicating vessels due to bronchial vasomotor activity can be transmitted to the lesser circuit (I. de B. Daly, 1956). In this connexion it has been shown by Bernthal (1934, 1938) that stimulation of the carotid body chemoreceptors causes reflex vasoconstriction in the *systemic* circulation. This finding has been confirmed in the present experiments in that chemoreceptor stimulation invariably caused an increase in cerebral perfusion pressure and in the pressure in the remaining parts of the perfused systemic circulation (Figs. 3*A*, *C*, 4*A*, *C*). These changes in systemic perfusion pressure are accompanied by similar changes in bronchial arterial pressure, and probably also in bronchial vasomotor tone because the bronchial circulation is part of the systemic circulation.

That a rise in systemic (bronchial) arterial pressure *per se* affects pulmonary vascular resistance is shown in Fig. 4. In *A* and *C*, chemoreceptor stimulation caused a rise in systemic (bronchial) arterial perfusion pressure and in pulmonary arterial pressure. When, however, a similar test was carried out while the systemic blood pressure was compensated, a fall in pulmonary arterial pressure occurred (*B*). This suggests that the increase in bronchial arterial pressure causes a passive rise in pulmonary vascular resistance (Berry & I. de B. Daly, 1931). On the other hand, the reduction in pulmonary vascular resistance occurring in response to chemoreceptor stimulation at *constant* systemic (bronchial) arterial pressure cannot be attributed to an effect on the pulmonary vascular bed proper because this has been shown to cause a rise in pulmonary vascular resistance. Thus, by inference, the reduction in pulmonary vascular resistance (Fig. 4*B*) is due to a reflex change in bronchial vascular resistance. The balance between these two passive mechanisms on the one hand, and the active reflex response of the pulmonary vascular bed proper on

the other, probably varied from one experiment to another, and if so would account for the variable pulmonary vascular resistance responses which occur on stimulation of the chemoreceptors under conditions in which blood circulates through the bronchial vascular system (Table 3).

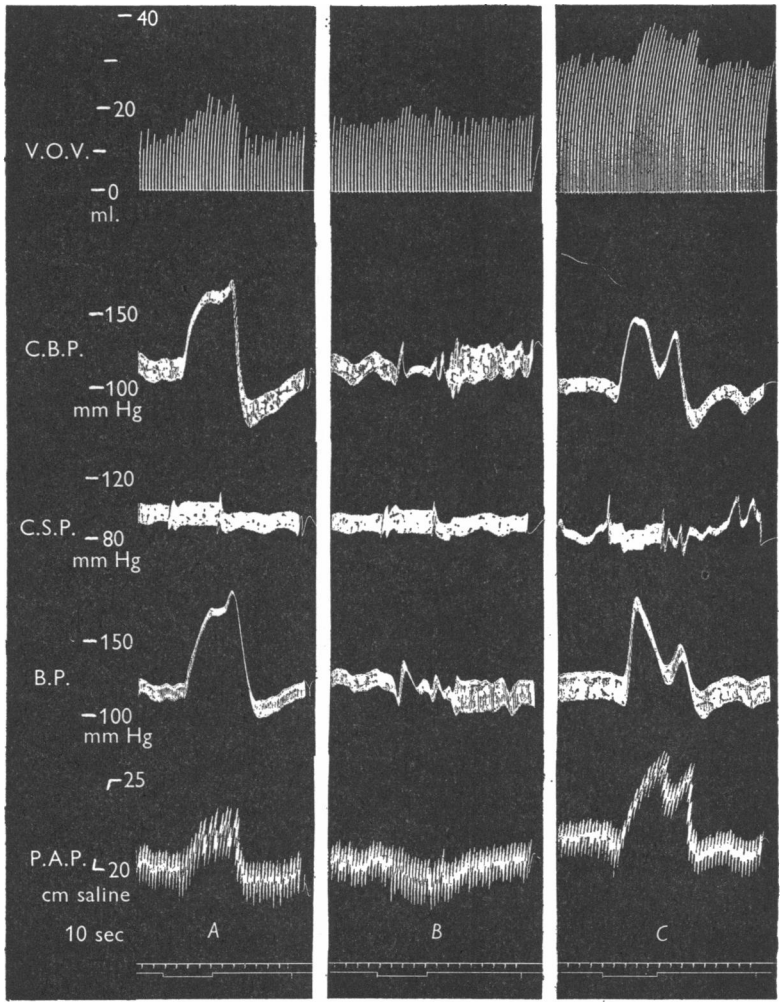

Fig. 4. Dog, ♀, 12 kg. Morphine-chloralose. 'Vasosensory controlled perfused living animal' preparation. The aortic arch was supplied with blood by the same pump as that perfusing the cerebral circulation (Fig. 2*A*). In *A*, *B* and *C*, the carotid body chemoreceptors were stimulated temporarily by venous blood from the right auricle of donor dog No. 1. In *B*, the mean pressures in cerebral circulation, the aortic arch and the vascular bed of the aorta beyond the origin of the left subclavian artery were maintained constant. Note reversal of the response of the pulmonary arterial pressure in *B*.

Changes in rate of the beating auricle

In several experiments the right auricle continued to beat for several hours after beginning perfusion of the whole animal. It was found that stimulation of the carotid body chemoreceptors slowed the rate of beating and occasionally stopped the auricle. These effects occurred at constant carotid sinus and aortic arch pressures and when the pressure in the vascular bed of the descending aorta was maintained constant, but were abolished by division of both cervical vagosympathetic nerves. These results provide further evidence that the primary cardiac reflex effect of stimulation of the carotid bodies is to slow the heart (see Bernthal, Greene & Revzin, 1951; Daly & Daly, 1957*b*; M. de B. Daly & Scott, 1958, 1959).

DISCUSSION

Aviado, Ling & Schmidt (1957) found in anaesthetized dogs that inhalation of 5% or 10% O_2 in N_2 caused an increase in pulmonary arterial pressure in an innervated, blood-perfused lung-lobe preparation, ventilated independently of the other lobes. They concluded that this response was due, at least in part, to reflex pulmonary vasoconstriction initiated by hypoxic stimulation of the carotid and aortic chemoreceptors. Their claim is based on the indirect evidence that the rise in pulmonary arterial pressure occurring in response to hypoxia is abolished by division of the carotid sinus and aortic nerves. This means that the responses of the lung blood vessels before and after denervation were obtained on a different 'background' because the influence of the carotid sinus and aortic arch baroreceptors on the pulmonary blood vessels had been excluded as well (Daly & Daly, 1959). Furthermore, their interpretation that the pulmonary arterial perfusion pressure responses to hypoxia were due entirely to active nervous effects on the pulmonary blood vessels themselves cannot, in our opinion, be upheld, since neither passive bronchomotor effects nor the mechanical effects of changes in the volume of the thorax or heart on the pulmonary vascular resistance were excluded. In this connexion it should be mentioned that hypoxia of the whole animal causes movements of the thorax and diaphragm which in turn lead to passive changes in pulmonary vascular resistance. Unless these movements are prevented by widely opening the thorax and/or by administering a neuromuscular blocking drug, their effects on pulmonary vascular resistance cannot readily be distinguished from those due to active nervous influences. Another mechanism passively affecting pulmonary vascular resistance receives no mention in their paper, namely, haemodynamic changes in the bronchial vascular system. It is not clear from the description of their experiments whether or not the bronchial circulation to the test lung-lobe was intact. If intact, the observed pulmonary arterial perfusion pressure responses would have been complicated by the passive effects of the bronchial circulation which we have already described. On the

other hand, if the bronchial circulation in their experiments had been interrupted, and we suspect this may have been so since they tied the bronchospirometric tube securely into the bronchus of the perfused lung-lobe, which would include the bronchial arteries, then we are at a loss to understand how the functional activity of the nerves to the lungs remained unimpaired, since in our experience active pulmonary vasomotor nerve activity in the dog is dependent upon the integrity of the bronchial circulation (I. de B. Daly & von Euler, 1932; I. de B. Daly, 1956). It is felt that these points are worthy of mention because the pulmonary vascular bed is subjected to so many passive effects which must be eliminated before a response to a given stimulus can be interpreted as an active nervous effect on the pulmonary vascular bed proper. Thus, in the entire animal stimulation of a specific type of sensory receptor may lead to reflex cardiac, vasomotor, respiratory and humoral effects. Any one of these may in turn produce secondary responses by various mechanisms such as stimulation of other receptors, for example those situated in specific areas of the cardiovascular system, or a central action due to changes in cerebral blood gas composition or a direct peripheral effect of secreted hormones. In this way the initiation of one reflex may lead to a complex chain of biological events, so making the analysis of the primary reflex response a matter of some difficulty.

In the 'vasosensory controlled perfused living animal' the problem has been simplified in that secondary reflex effects on the pulmonary vascular bed arising from receptors in the heart and systemic circulation have been eliminated, thereby enabling the examination of the primary reflex vascular responses to stimulation of the carotid body chemoreceptors. It should be mentioned that since the lungs were under controlled ventilation, the phrenic nerves cut and the chest widely opened, any respiratory reflexes which stimulation of the chemoreceptors may have elicited would have been ineffective in modifying the primary vascular responses, with the possible exception of an irradiation of impulses from the respiratory 'centre' to the vasomotor 'centre'. Although in this paper we have described the effects of stimulation of the carotid body chemoreceptors on the pulmonary vascular bed under such controlled conditions, it is evident that the primary reflex responses of any vascular territory resulting from stimulation of other types of receptor can be analysed in a similar way (Daly & Daly, 1959).

We have confirmed our previous findings (Daly & Daly, 1957*b*) that stimulation of the carotid body chemoreceptors usually causes a reduction in pulmonary vascular resistance provided the bronchial circulation is intact. A similar response has never been observed in the absence of the bronchial circulation; on the contrary, an increase in vascular resistance has always occurred, indicating that the direct reflex effect on the pulmonary vascular bed proper is vasoconstriction.

Evidence has been presented as to why, in those experiments in which blood is flowing through the bronchial vascular system, stimulation of the carotid bodies may cause a rise or a fall in pulmonary vascular resistance. These results are of interest in relation to those obtained by Daly & Daly (1957*b*). In their experiments they found that the fall in pulmonary vascular resistance resulting from stimulation of the carotid body chemoreceptors was accompanied by only slight changes in systemic (bronchial) arterial pressure. The counterpart of these results is found in the present experiments under conditions in which the chemoreceptors were stimulated while the systemic (bronchial) arterial pressure was held constant.

The most likely explanation of this fall in pulmonary vascular resistance is a reflex alteration in resistance of the bronchial arteries and their communicating vessels leading to a redistribution of blood between the bronchial and pulmonary vascular systems. When, however, large rises in systemic (bronchial) arterial pressure are allowed to occur, as in some of the present experiments, a rise in pulmonary vascular resistance takes place due to a passive mechanism, as was demonstrated by Berry & I. de B. Daly (1931).

There is one further point which should be mentioned. We were not entirely satisfied that in every test the rise in pulmonary arterial perfusion pressure which accompanied a reflex increase in systemic arterial pressure, due to chemoreceptor stimulation, was the result of haemodynamic changes in the bronchial circulation. Daly & Daly (1957*a*) suspected that the distension of the aorta due to the rise in systemic blood pressure might compress the pulmonary artery and its branches and thus produce a rise in pulmonary *arterial* resistance. Although in the present experiments we took every precaution to eliminate this complication, it was not possible to do so in every experiment owing to the complex nature of the 'plumbing' in the thorax. This possibility, however, does not militate against what appears to us unequivocal evidence that, at zero bronchial arterial pressure, and hence during collapse of the thoracic aorta—and in some experiments the aortic arch as well—carotid chemoreceptor stimulation caused a reflex constriction of the pulmonary vascular bed proper.

In regard to the nervous pathways mediating the changes in pulmonary vascular resistance, Daly & Daly (1957*b*) reported that, *when the bronchial circulation was intact*, the *decrease* in pulmonary vascular resistance occurring on stimulation of the carotid chemoreceptors persisted after destruction of the upper thoracic sympathetic outflow but was abolished by vagotomy or by atropine. In the light of our present experiments, which demonstrate the participation of the bronchial circulation in the production of this *decrease* in vascular resistance, our previous results could be interpreted as indicating a reflex vasodilatation of the bronchial circulation, since Bruner & Schmidt (1947) demonstrated the presence of bronchial vasodilator fibres running in

the vagus nerves. If this explanation is the correct one, then it is difficult, in view of our present findings, to understand why atropine or vagotomy should have merely abolished the decrease in pulmonary vascular resistance in response to chemoreceptor stimulation and not have reversed it to an increase in vascular resistance. No satisfactory explanation for this can at present be offered. The haemodynamic relationships between the bronchial and pulmonary vascular systems are complex and not yet fully understood. In this connexion, both the bronchial and possibly also the pulmonary circulation each have a dual innervation (Bruner & Schmidt, 1947; I. de B. Daly & Hebb, 1952). Thus an alteration of the impulse discharge in either the vagal or sympathetic fibres to the lungs may affect the pulmonary vascular resistance passively through an action on the bronchial circulation, or actively by altering the calibre of the pulmonary blood vessels themselves. It is clear that further work is necessary to elucidate these mechanisms but it should be stressed again that whatever their nature, reflex changes both in bronchial arterial pressure and in bronchial vascular resistance have been ruled out in our experiments as a cause of the increase in pulmonary vascular resistance occurring on stimulation of the carotid body chemoreceptors.

In one experiment of the present series we were able to demonstrate that the reflex vasoconstriction of the pulmonary vascular bed proper was dependent on the integrity of the upper thoracic sympathetic outflow. This finding is of interest because it could be inferred that, under certain conditions, the tone of the pulmonary blood vessels themselves is maintained by the sympathetic nervous system. These conditions pertain to those in which there is stimulation of the carotid body chemoreceptors, namely, hypoxia, hypercapnia and haemorrhage (for literature, see Heymans & Neil, 1958).

SUMMARY

1. A perfused whole animal preparation has been devised in which the vasosensory areas of the carotid bifurcations and of the aortic arch, the brain, the remainder of the systemic circulation and the lungs were separately perfused. In this preparation it is possible to determine the reflex response in any given vascular territory occurring on stimulation of a specific type of receptor uncomplicated by the effects of secondary reflex and humoral mechanisms initiated by concomitant effects in other organs of the body as a result of the same stimulus. We propose to call this the 'vasosensory controlled perfused living animal' preparation.

2. When blood is flowing through the bronchial vascular system, stimulation of the carotid body chemoreceptors by venous or by hypoxic blood causes either an increase, a decrease or no change in pulmonary vascular resistance as indicated by the change in the pressure gradient across the pulmonary vascular bed at constant pulmonary arterial blood flow.

3. When the tests were repeated during temporary interruption of blood flow through the bronchial circulation, chemoreceptor stimulation invariably caused an increase in pulmonary vascular resistance.

4. This response occurred independently of appreciable changes in lung 'hindrance', in left auricular pressure and in pressure in the other parts of the systemic circulation.

5. It is concluded that carotid chemoreceptor stimulation causes constriction of the pulmonary vascular bed proper but that this response may be masked by a passive mechanism, namely, haemodynamic events taking place in the bronchial circulation thereby altering the distribution of blood between the bronchial and pulmonary vascular systems.

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